

WHAT IS CLAIMED IS:

1. A method for producing mammalian
5 proteins comprising:
growing mammalian secondary expression host
cells comprising multiple copies of an amplifiable
region comprising a target gene heterologous to said
secondary expression host and expressing a protein of
10 interest and an amplifiable gene, whereby said target
gene is expressed and said protein is produced;
wherein said secondary host expression cells
are produced by the method comprising:
transforming primary mammalian cells
15 comprising said target gene with a construct
comprising an amplifiable gene and at least one
flanking region of a total of at least about 150 bp
homologous with a DNA sequence at the locus of the
coding region of said target gene to provide
20 amplification of said target gene, wherein said
amplifiable gene is at a site which does not
interfere with the expression of said target gene,
whereby said construct becomes homologously integrated
into the genome of said primary cells to define an
25 amplifiable region;
selecting for primary cells comprising said
construct by means of said amplifiable gene or other
marker present in said construct;
isolating DNA portions of said genome from
30 said primary cells, wherein said portions are large
enough to include all of said amplifiable region;
transforming secondary expression host cells
with said primary cell DNA portions and cloning said
transformed secondary expression host cells to
35 produce clones of said secondary expression host
cells differing in said DNA portions present in said
secondary expression host cells;

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selecting clones of said mammalian secondary
expression host cells comprising said amplifiable
region; and

5 amplifying said amplifiable region by means
of an amplifying agent, wherein said amplifying is
prior to said isolating or after said selecting and
prior to said growing.

2. A method according to Claim 1, wherein
said amplifiable gene is a mammalian DHFR gene.

10 3. A method according to Claim 1, wherein
said portions are metaphase chromosomes.

4. A method according to Claim 1, wherein
said portions are restriction fragments.

15 5. A method according to Claim 1, wherein
said primary cells are human cells.

6. A method according to Claim 5, wherein
said human cells are fibroblast cells.

20 7. A method according to Claim 1, wherein
said construct comprises a biocidal marker providing
resistance to a biocide for said primary host cells.

8. A method for producing mammalian
proteins comprising:

25 transforming mammalian primary mammalian
cells comprising said target gene with a construct
comprising an amplifiable gene and at least one
flanking region of at least about 150 bp homologous
with a DNA sequence within 50 kb of the coding region
of said target gene, wherein said amplifiable gene is
at a site which does not interfere with the
30 expression of said target gene, whereby said
construct becomes homologously integrated into the
genome of said primary cells to define an amplifiable
region comprising said amplifiable gene and said
target gene in said genome;

35 selecting for primary cells comprising said
construct by means of said amplifiable gene or other
marker present in said construct;

isolating DNA portions of said genome from
said primary cells, wherein said portions are large
enough to include all of said amplifiable region;

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5 transforming mammalian secondary expression
host cells with said primary cell DNA portions,
wherein said secondary expression host cells are of a
different species from said primary host cells, and
cloning said transformed secondary expression host
cells to produce clones of said secondary expression
10 host cells differing in said DNA portions present in
said secondary expression host cells;

selecting clones of said mammalian secondary
expression host cells comprising said amplifiable
region;

15 amplifying said amplifiable region by means
of an amplifying agent, wherein said amplifying is
prior to said isolating or after said selecting; and

growing said secondary expression host cells
comprising multiple copies of said amplifiable region,
20 whereby said target gene is expressed and said protein
is produced.

9. A method according to Claim 8, wherein
said amplifying is with said secondary expression host
cells.

25 10. A method according to Claim 8, wherein
said primary cells are human cells.

11. A method according to Claim 10, wherein
said human cells are diploid fibroblast cells.

30 12. A method according to Claim 8, wherein
said amplifiable gene is a mutated DHFR gene having a
higher Km than the wild-type gene.

13. A method according to Claim 12, wherein
said secondary host expression cell is DHFR deficient.

35 14. A method according to Claim 8, wherein
said construct further comprises a marker gene
separated from said amplifiable region by an
homologous flanking region.

isolating DNA portions of said genome from
said primary cells, wherein said portions are large
enough to include all of said amplifiable region;

transforming mammalian secondary expression
5 host cells with said primary cell DNA portions,
wherein said secondary expression host cells are of a
different species from said primary host cells, and
cloning said transformed secondary expression host
cells to produce clones of said secondary expression
10 host cells differing in said DNA portions present in
said secondary expression host cells;

selecting clones of said mammalian secondary
expression host cells comprising said amplifiable
region; and amplifying said amplifiable region by
15 means of an amplifying agent, wherein said amplifying
is either prior to said isolating or after said
selecting.

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20 21. A method according to Claim 20, wherein
said amplifying is with said secondary expression host
cells.

22. A method according to Claim 20, wherein
said primary cells are human cells.

23. A method according to Claim 22, wherein
said human cells are diploid fibroblast cells.

25 24. A method according to Claim 20, wherein
said amplifiable gene is a mutated DHFR gene having a
higher Km than the wild-type gene.

25. A method according to Claim 24, wherein
said secondary host expression cell is DHFR deficient.

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